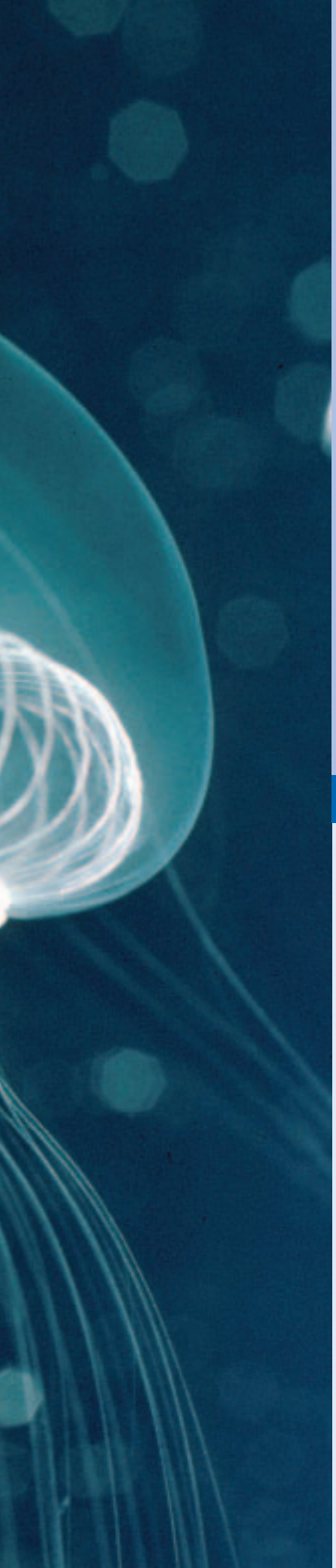
UNIT

C

Cycling of Matter in Living Systems

A reflected light image of the jellyfish species in which the gene for the luminescent green fluorescent protein (GFP) was discovered. **Inset:** Genetically engineered mice, carrying the gene for GFP, luminesce under ultraviolet light.



In this unit, you will cover the following ideas:

C 1.0 **Our current understanding of the cell is due in part to developments in imaging technology.**

- **C1.1** A Window on a New World
- **C1.2** Development of the Cell Theory
- **C1.3** Developments in Imaging Technology and Staining Techniques
- **C1.4** Cell Research at the Molecular Level

C 2.0 **Living systems are dependent upon the functioning of cell structures and organelles.**

- **C2.1** The Cell as an Efficient, Open System
- **C2.2** The Role of the Cell Membrane in Transport
- **C2.3** Applications of Cellular Transport in Industry and Medicine
- **C2.4** Is Bigger Better?

C 3.0 **Plants are multicellular organisms with specialized structures.**

- **C3.1** Cells, Tissues, and Systems
- **C3.2** The Leaf and Photosynthesis
- **C3.3** The Leaf Tissues and Gas Exchange
- **C3.4** Transport in Plants
- **C3.5** Control Systems

Focus on the Nature of Science

In this unit, you will learn how cell structures and organelles function to carry out the life processes in living organisms. You will discover how technological advancements have improved our understanding of cell structure and function.

Useful questions to guide you in your study include:

1. How did the cell theory replace the concept of "spontaneous generation" and revolutionize the study of life sciences?
2. How do single-celled organisms carry out life functions?
3. How do plants use specialized cells and processes to accomplish the same functions as a single cell, but on a larger scale?
4. How does imaging technology further our understanding of the structure and function of cells?

At the end of the unit, you may be asked to do these tasks:

Case Study Help Wanted

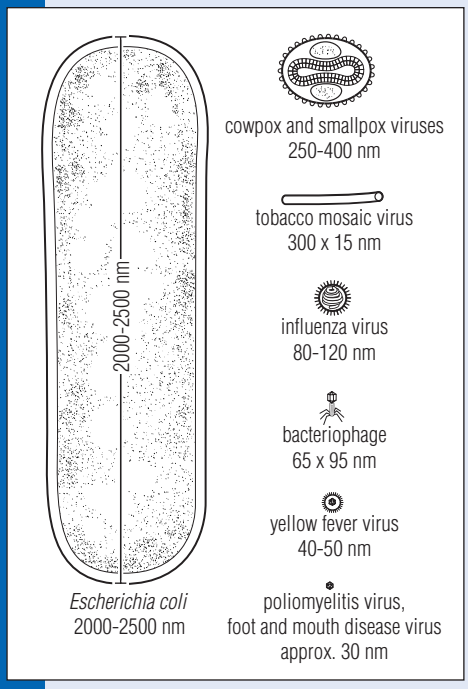
In this case study, you will research engineering applications of transport systems in cells. You will prepare a presentation on a specific area of research to make the case for its application in industry or medicine.

Project The Impact of Environmental Factors on Plant Function

In this project, you will develop a proposal to investigate the question: Are plant cells able to respond to changing levels of carbon dioxide? You will state a question and hypothesis, identify relevant variables, and design an experimental procedure.

Exploring

■ Municipalities are responsible for maintaining the quality of drinking water provided through water treatment facilities.



■ Micro-organisms like bacteria and viruses can range in size from 10 µm to just a few nanometres.

In May 2000, the residents of Walkerton, Ontario, suffered Canada's worst case of water contamination. Most types of the bacterium, *Escherichia coli*, are harmless; several types are even found in the intestines of humans and animals. However, *E. coli* strain O157:H7, the strain that entered the water supply of this small town, produces a powerful toxin that can cause serious illness. Seven people in Walkerton died as result of ingesting *E. coli* strain O157:H7 bacteria. These deaths brought renewed national attention to the importance of clean, safe drinking water. Victims of the Walkerton contamination were offered an experimental drug developed at the University of Alberta by Dr. Glen Armstrong, called "Synsorb," which is able to absorb *E. coli* toxins in the human digestive tract.

How do we protect against water contamination like that experienced in Walkerton? Environment Canada identifies good drinking water as being free of disease-causing organisms, harmful chemical substances, and radioactive matter. The water should have a good taste and be odourless and clear. Maintaining clean drinking water is an important task and not an easy one. Bacteria, viruses, and other physical, chemical, and biological contaminants may be washed into lakes, rivers, streams, reservoirs, and groundwater, which are all common sources of drinking water for cities and towns. Municipalities are responsible for maintaining the quality of drinking water provided to their residents. In order to accomplish this task, water treatment facilities usually have a pre-filtering process to eliminate large contaminants, and then a full-scale water purification process to ensure that the water is potable (fit for drinking). This process includes treatment to solidify impurities, filtration through several layers of sand or charcoal, and the addition of chlorine disinfectant.

There are other, more expensive, methods of purifying water that use little or no chemical disinfectant and depend on what have become known as "membrane technologies." The membrane is a synthetic fibre barrier that contains microscopic holes or pores through which pure water can pass, but physical and biological contaminants cannot. You may have heard of "reverse osmosis" water purification systems, in which water is passed through a series of membranes at very high pressure. This is one of many types of membrane technologies that are being developed.

Membrane technologies are used not only in water purification, but also in chemical, petrochemical, pharmaceutical, food, agricultural, environmental, and biotechnology industries. What you may not know is that the use of membranes, to select which substances can pass through, is modelled on the way in which plant and animal cells transport materials. Technology enables the study of natural systems, but the reverse is true, as the study of natural systems can help us to identify and develop synthetic systems to perform similar functions at high efficiency. Some of these systems and their applications in industry will be explored in this unit.

Activity C1

QuickLab

Student Reference 5

Pore Size in Various Materials

The pore size of a material will determine what can pass through it. This concept is important in water filtration systems. If a product cannot eliminate bacteria and viruses, additional purification methods may be needed.

Purpose

To examine the relative pore size of different materials and to relate pore size to the ability to exclude micro-organisms

Materials and Equipment

cloth samples (e.g., linen, cheesecloth, unbleached muslin, polyester, or other materials such as waterproofed fabric)
paper samples (e.g., filter paper, paper towel)
scissors
water
dropper
microscope slides
dissecting microscope (optional) or magnifying glasses
small, flat, transparent metric ruler

CAUTION: Use proper technique when handling microscopes and magnifying glasses. Handle glass slides carefully.

Procedure

1. Cut a small square (of side about 1.0 cm) of each of the cloth and paper samples provided by your teacher.
2. Using a dropper, place several drops of water on a microscope slide. Add one square of cloth to the water to make a flattened wet mount.
3. Examine the square of cloth through the dissecting microscope or by using the magnifying glass. Place a ruler on the wet mount and, using the magnifying glass, count or estimate the number of pores present in the cloth along a line 0.5 cm as shown on the ruler.
4. Draw a diagram of the weave of the cloth or the surface of the paper.
5. Repeat your observations for two other samples. Compare your results with those of another student who has used different materials.

Questions

1. Compare the weave and pore size of the different materials you observed. Which material would be most efficient as a filtration membrane? Explain your choice.
2. What is the relation between pore size and filtration ability? Rank the materials supplied to the class in order of their ability to filter out particles.
3. Consider the pore size of each type of filter system listed across the top of the table shown below. Complete the table to indicate which of the micro-organisms listed in the left hand column can be excluded by which filter. Use + to indicate that a filter can prevent an organism from passing through it and – to indicate that it cannot.

Filters	Granulated Activated Carbon (20 µm)	Carbon Block (0.5 µm)	Micro Filtration (0.1–1.0 µm)	Ultra Filtration (0.001–0.01 µm)
Paramecium caudatum (180–300 µm)				
Giardia lamblia (8–12 µm)				
Escherichia coli (2.0–2.5 µm)				
smallpox virus (0.25 µm)				
poliomyelitis virus (0.03 µm)				

4. Explain why simple filtration alone may not be sufficient to maintain a safe water supply.

UNIT C

1.0

Key Concepts

In this section, you will learn about the following key concepts:

- microscopy and the emergence of cell theory

Learning Outcomes

When you have completed this section, you will be able to:

- trace the development of the cell theory: all living things are made up of one or more cells and the materials produced by these, cells are functional units of life, and all cells come from pre-existing cells
- describe how advancements in knowledge of cell structure and function have been enhanced and are increasing as a direct result of developments in microscope technology and staining techniques
- identify areas of cell research at the molecular level

Our current understanding of the cell is due in part to developments in imaging technology.

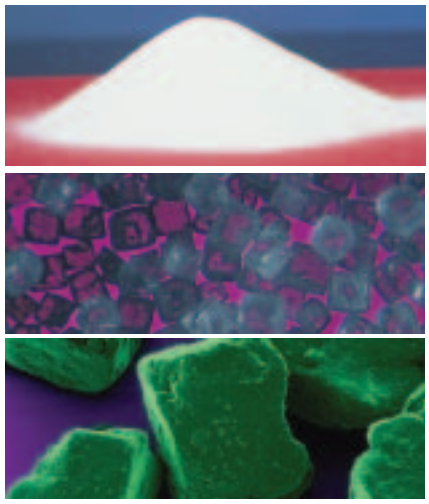


FIGURE C1.1 Compare the detail seen in these three images of table salt.

Look at the images of grains of salt seen through the naked eye, under 400× magnification, and using an electron microscope (Figure C1.1). Describe "salt" as seen through each image.

Microscopes are used in many areas of our lives. Forensic scientists solve crimes through the microscopic analysis of hair, fluids including blood, body tissues, fibres, soil, and DNA. Crop diseases and pathogens in food, water, and air can all be detected through the study of microorganisms under the microscope. Tiny defects in the metal of bridges and airplanes are found with the use of microscope technology. Diseases like malaria and tuberculosis can be prevented and treated because of information obtained through the use of microscopes.

Microscope technology even affects surgery. Today, many operations are performed using new instruments and a technology known as laparoscopic or keyhole surgery. In this procedure, the surgeon makes one to three tiny incisions, and then inserts a fibre-optic light, scalpel, and other surgical tools through a thin tube. A baton-like instrument fitted with a magnifying eyepiece with a tiny video camera provides a highly magnified view of the surgical area on a television screen. There are many benefits to laparoscopic surgery; the patient generally loses less blood, experiences less pain, has a faster recovery time, and the tiny incisions produce less scarring. This technique is used in performing knee surgery, removing gallstones, hernia operations, and a ground-breaking procedure called "closed-heart bypass surgery," in which a blocked cardiac artery is repaired using robotic and computer-assisted tools, without the need to open the chest cavity.

In this section, you will learn how our understanding of the cell and its structure and function has developed over time, and has depended on advancements in imaging technology. You will also be introduced to areas of cell research that have an impact on the daily lives of many people, whether they know it or not.

C1.1 A Window on a New World

Throughout history, people have been intrigued by the structure of living things. Our current understanding of life processes is the result of developments that date from the time of Aristotle, a Greek philosopher of the 4th century B.C. As you know from earlier studies, the inquiry process for science involves a cause-and-effect question that leads to a hypothesis, and an experiment to test the hypothesis. To know what questions to ask, a scientist must first carefully observe the system being studied. The early Greeks were primarily philosophers, preferring to think out possible answers to questions rather than testing their ideas, but Aristotle followed a pathway of accurate observation and record making, followed by reasoning and interpretation. Aristotle made careful observations and descriptions of more than 500 animal species and set up a classification system based on his observations. His approach was a fore-runner of methods used by modern scientists. It was not until the invention of the microscope, however, that scientists were able to see and begin to understand the building blocks of living things.

Early Microscopes and Microscopists

A window was opened on the microscopic world when the development of the theory of optics and the discovery of the magnifying properties of lenses allowed scientists to study objects smaller than could be seen with the human eye. It is believed that Hans and Zacharias Janssen, Dutch lens-makers, invented the microscope in about 1595, using a two-lens system of an eyepiece, or ocular lens, and an objective lens (Figure C1.2(a)). This would have been the first "compound" microscope, meaning that it made use of more than one lens to magnify objects. The Janssens' microscope had a magnifying power of approximately 20×.

By 1665, Robert Hooke in England was using a hand-made microscope (Figure C1.3) that had a three-lens system (Figure C1.2(b)). The illumination was a beam of light concentrated on the specimen by passing the light through a water-filled glass flask. In that year, Hooke published his *Micrographia* containing 38 illustrations of plant, animal, and non-living objects viewed through the microscope. Hooke was interested in gaining information about the structure of cork. It was not understood at the time why cork had its unusual properties: it was light-weight; could float on water; was firm, yet compressible under force. He examined thin slices and saw many empty chambers which he called "cells," as shown in Figure C1.4.

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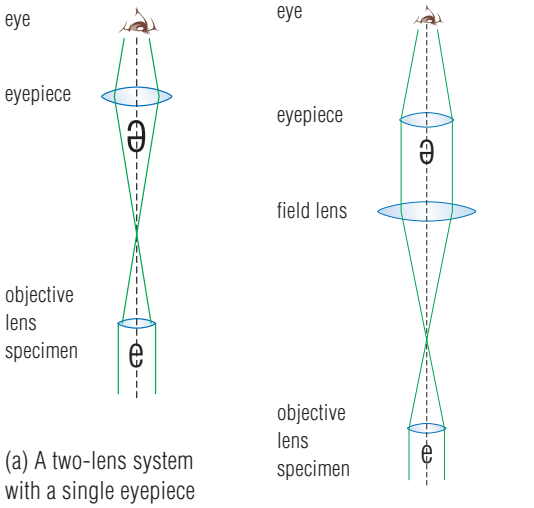
The word "cell" is derived from the Latin word *cellula*, meaning "small compartment." Why do you think Robert Hooke coined the word "cell"?



(a) A two-lens system with a single eyepiece (ocular) lens

(b) Hooke's three-lens system has two lenses in the ocular. The field lens passes light rays in a straight line to the eyepiece. This allows better conservation of light from the object.

FIGURE C1.2 Lens systems in the light microscope

FIGURE C1.3 The elegant compound microscope made for Robert Hooke in 1660

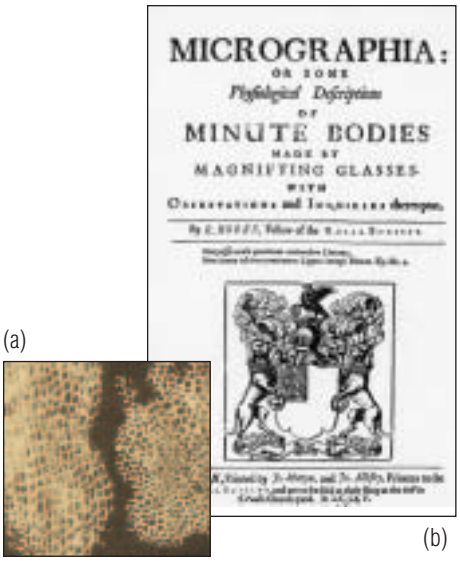


FIGURE C1.4 Hooke's drawings of cork cells (a) appeared in his *Micrographia* of 1665 (b).

The cork therefore consisted primarily of air pockets surrounded by a thin mesh of fibre. He did not know then that these tiny chambers were the remnants of living cells, the simplest functional units of life.

At about the same time, a Dutch businessman, Antoni van Leeuwenhoek, using only a simple single-lens microscope rather like a magnifying glass, was the first to see the movement of different types of single cells that we now know as bacteria, sperm, and unicellular protozoa. These were the first observations of individual free-living cells surviving as independent systems. Van Leeuwenhoek made careful drawings of his discoveries and named them "animalcules." His microscopes were small, probably about the size of his palm, and the lens was held directly at the eye. This system was difficult to use. However, because of van Leeuwenhoek's skill in making the tiny lenses for his microscopes, he was able to produce higher magnifications than those of the compound microscopes of the day (up to 250×). In bright light, this allowed for clear observation of cell structure and movement. Light microscopes continue to be important instruments to the cell biologist because they allow the scientist to see the movement of living cells. Hooke and van Leeuwenhoek both recorded their observations in detail and made them available to others by publishing through the Royal Society of London.

Improvements in Lens Technology

Van Leeuwenhoek's success was mainly due to the quality of his lenses. Early compound microscopes were less efficient than van Leeuwenhoek's single lenses because the images produced by the compound microscopes were often blurry, with a halo of light around the object. These image problems were the result of the light being scattered as it passed through the different lenses. During the 18th century, a combination of lenses called an achromatic lens was developed to control the halo and improve the amount of detail that could be seen.

*re*SEARCH

Sometimes, "scientists" are thought of as stereotyped old, eccentric brains in white lab coats. In reality, the people responsible for major scientific discoveries often have interesting and even colourful stories to their lives. Find out about the people who were involved in the development of the microscope. Write a biographical paragraph about one of these personalities. Try to capture their interests and pursuits, including but not limited to contributions to science. Begin your search at

www.pearsoned.ca/school/science10

Skill Practice — Calculating Magnification

In Activity C2 Estimating an Object's Size with the Microscope, you will use the compound microscope to estimate the size of objects. This Skill Practice will give you the chance to review how magnification is calculated. In a modern compound microscope, the power of the objective lens and the power of the eyepiece are multiplied to give the magnifying power or **magnification** of the system. What is the magnification of a system that has a 4× objective lens and a 10× eyepiece ?

magnification = (power of objective lens) (power of eyepiece)
= (4)(10)×
= 40×

What is the magnification if the following combinations of lenses are used?

a) a 2.5× low-power objective lens and a 10× eyepiece

b) a 100× high-power objective lens and a 10× eyepiece

Activity C2

Inquiry Lab

Student Reference 8

Required Skills
- Initiating and Planning
- Performing and Recording
- Analyzing and Interpreting
- Communication and Teamwork

Estimating an Object's Size with the Microscope

A compound light microscope magnifies specimens. The magnification depends on the combination of lenses used. It is interesting and informative to view objects under a microscope, but it is often difficult to know the actual size of the object being observed. Magnification causes us to lose our perspective on size. In this lab, you will learn how to estimate the size of an object by comparing it with something you already know—the diameter of the **field of view**.

The Question

How can the compound microscope be used to estimate the size of microscopic specimens?

The Hypothesis

If the diameter of the field of view is known, then the size of an object can be estimated.

Materials and Equipment

- compound light microscope
- flat transparent metric ruler small enough to be positioned on the microscope stage
- prepared slides: animal cells, plant cells, unicellular organisms
- unlined paper
- pencil
- mathematical compass

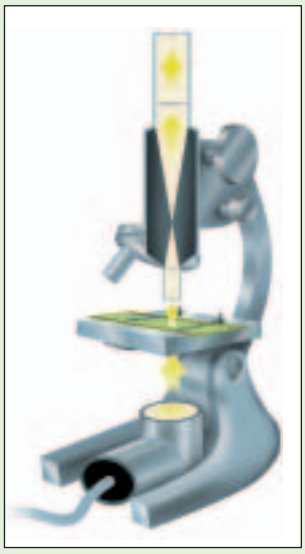


FIGURE C1.5 Set-up for measuring the diameter of the field of view

CAUTION: Observe proper technique with the microscope and slides to ensure safe handling of equipment.

Procedure

1. Review the proper handling and use of the microscope in Student Reference 8: The Compound Light Microscope.
2. Set up your microscope and place a transparent metric ruler on the stage so that it covers about half of the stage as shown in Figure C1.5.
3. Observe the ruler under low power. The **field of view** is the entire area that you see when you look through the microscope. Move the ruler so that you are measuring the diameter (width) of the low-power field of view from left to right and set one of the millimetre divisions at the edge of the field of view as shown in Figure C1.6.

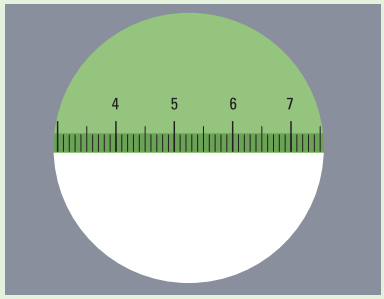


FIGURE C1.6 Move the ruler so that you can measure the diameter of the field of view. Line up a millimetre mark at the edge of the circle.

4. Create a data table in your notebook like the one below. Remember to give your table a title. Record the magnification for each power.

Field	Magnification	Field Diameter (mm)	Field Diameter (µm)
low power			
high power			

Measure the diameter of the low-power field to the nearest tenth of a millimetre. Record this measurement in the table in your notebook. Convert the diameter from millimetres to micrometres. Record in the table. Remember, 1 mm = 1000 µm.

5. You cannot measure the diameter of the high-power field of view using the procedure outlined above because it is less than one millimetre, but you can use the following ratio to calculate the field diameter under high power. This relationship between the field of view and the degree of magnification is represented in the following formula:

$$\frac{\text{high-power field diameter}}{\text{low-power field diameter}} = \frac{\text{low-power magnification}}{\text{high-power magnification}}$$

Show your work. Record the high-power field diameter in millimetres and micrometres.

6. Examine a prepared slide of an animal or plant specimen through the low- and high-power objective lenses. Draw what you see in the field of view on low power. Calculate the **scale** of your drawing by comparing the diameter of the circle in your drawing with the field diameter that you obtained in step 5. For example, if the field diameter of the low-power objective was 3 mm and the diameter of the circle on the drawing was 3 cm (30 mm), the scale of the drawing would be 10:1. Refer to Student Reference 8 for a discussion of scale drawings.

7. Estimate the size of objects you view under the microscope by comparing them with the diameter of the field of view. An organism that takes up one-half of a field of view that is 500 µm in diameter, has a size of about one-half of 500 µm, or 250 µm.

8. Obtain prepared slides of various organisms and practise estimating their lengths and/or widths. Record the information in a data table.

9. Return your microscope and slides to their proper storage locations once you have finished this activity.

Analyzing and Interpreting

1. How many times is the magnification increased when you change from the low-power to the high-power lens?

2. How many times is the field diameter decreased when you change from the low-power to the high-power lens?

Forming Conclusions

3. State how you would estimate the size of an object viewed under the high-power (40×) objective lens, if you were given the size of the field diameter, when using the low-power (4×) objective lens.

C1.1 Check and Reflect

Knowledge

1. Contrast the methods and results of Hooke and van Leeuwenhoek.
2. List ways that the investigations of Aristotle, Hooke, and van Leeuwenhoek were similar to modern scientific investigations.
3. What is the difference between a simple and a compound microscope?
4. What is the field of view when making observations through the light microscope?
5. Explain how to calculate total magnification when using a compound light microscope. Give an example.

Applications

6. Using the medium-power (10×) objective lens, a student measured the diameter of the field of view as 1.5 mm. Convert this diameter to micrometres.
7. Use the value you found in question 6 to calculate the field diameter when the student used the high-power (40×) objective lens.
8. A structure on a diagram measures 2.5 cm. The actual size of this structure is 0.5 mm. What is the scale of the diagram?

Extension

9. Using what you have learned about optics in previous science courses, research and explain the use of convex lenses in a compound light microscope.

C1.2 Development of the Cell Theory

Sometimes, scientists find that even with solid evidence to support new ideas, it is difficult to change traditional thought. Social pressure has an effect on the acceptance of scientific ideas and technological advancements. The attitudes and skills of scientific inquiry, including questioning, predicting, observing, and recording, are required to provide unbiased and factual information. Investigations must follow ethical guidelines, and results must be reproducible under controlled conditions. An example of the way that science, technology, and society are linked is found in the development of our current understanding of the way living cells function. The microscope provided the technology to explore the world of microscopic particles and organisms. It was then possible to obtain evidence for or against generally accepted opinions or theories about living things.

infoBIT

The theory of spontaneous generation was proposed by Aristotle in about 350 B.C.

Spontaneous Generation

The idea that life could emerge spontaneously from non-living matter was widely accepted from the time of the Romans through to the 19th century. Even during the time of Robert Hooke and Antoni van Leeuwenhoek, it was generally accepted that to produce mice, one simply had to put sweaty underwear and husks of wheat in an open jar. After about 21 days, the sweat and husks would combine and change the husks into mice. You may find this theory amusing, but how would you explain the presence of the mice in the jar?

In 1668, Francesco Redi, an Italian physician and poet, questioned the belief that maggots appeared spontaneously from raw meat. Instead, he believed that flies laid their eggs in the meat. Redi set up an experiment to test his hypothesis. He set out flasks containing raw meat, but some were sealed, some were covered in gauze, and some were open to the air. In this way, he controlled the access of flies to the meat. Maggots were found only in the flasks that were open and accessible to flies to lay their eggs (Figure C1.7). Despite the evidence, the idea of **spontaneous generation** continued to thrive.

In an effort to prove that living things could be produced from non-living matter, in 1745, English clergyman John Needham boiled chicken broth and put it in a flask and sealed it. Everyone accepted that boiling killed micro-organisms, since boiling was a common method of removing substances that would make one ill; however, in Needham's experiment, micro-organisms still appeared. Victorious, Needham suggested there was a **life force** that produced spontaneous generation. Lazzaro Spallanzani, an Italian priest and scientist, refuted Needham's claim and instead proposed that there were micro-organisms in the air that were responsible for the new growth. He repeated Needham's experiment but drew off the air in the flask. Nothing grew in the remaining broth. Critics suggested that all Spallanzani had shown was that air was required for spontaneous generation to occur. The theory of spontaneous generation continued to be accepted.

In 1859, the French Academy of Sciences announced a contest for the best experiment to prove or disprove spontaneous generation. The French

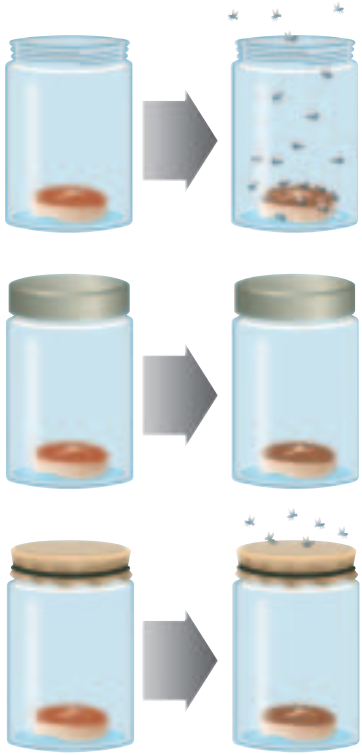


FIGURE C1.7 In Redi's experiment, the manipulated variable was access of flies to the meat.

chemist Louis Pasteur submitted the following experiment in 1864. He used the work of Needham and Spallanzani with an important change. Before boiling meat broth in a flask, Pasteur heated the neck of the flask and bent it into an "S" shape, as shown in Figure C1.8. Air could reach the broth, but micro-organisms and other particles would get caught in the S-bend. Nothing grew in this broth, but if the flask were tipped so that the broth reached the S-bend in the neck, moulds would later appear. The text on page 249 is taken from a translation of hand-written notes of a speech in French given by Louis Pasteur at the *Sorbonne Scientific Soiree* on April 7, 1864.

This text allows us to review the characteristics of a scientific experiment. Pasteur controlled his experiment in that he used the same broth, the same type of flasks, and the same light and temperature conditions. All of these—broth type, flask type, light, and temperature—are the **controlled variables**. Pasteur's **manipulated variable** was the access of dust to the flask; his **responding variable** was the ability to grow mould in the broth. In the experimental series in which he manipulated the access of dust to the flask, Pasteur had an experimental **control**, a part of the experiment in which the manipulated variable is not changed in any way from its normal condition. His experimental control was the flask in which dust had normal access to the broth after boiling, with the result that mould growth occurred.

His experimental treatments were:

a) to prevent the access of dust to the broth, resulting in evidence of no growth of mould;

b) to allow access of dust to the broth very briefly, resulting in evidence of mould growth.

Pasteur (Figure C1.9) provided strong evidence that spontaneous generation did not occur, but also that micro-organisms are found in the air. His work opened new doors to microbiology, immunology, and biochemistry, and gave credibility and new importance to the processes of conducting controlled experiments, maintaining detailed records of observations, and connecting results to conclusions. On the discipline of strict experimental tests, he commented: *"Imagination should give wings to our thoughts but we always need decisive experimental proof, and when the moment comes to draw conclusions and to interpret the gathered observations, imagination must be checked and documented by the factual results of the experiment."*

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FIGURE C1.9 Louis Pasteur, a French scientist who disproved the concept of spontaneous generation

FIGURE C1.8 Examples of Pasteur's flasks that did not become contaminated. The one in the middle is sealed; the others open but with an S-bend.

Pasteur Tackles Spontaneous Generation Theory

"Now suppose I decant a portion of this infusion of organic material into a long-necked flask, such as this one. If I boil it and allow it to cool, then in a few days, it will contain fully-developed moulds or infusoria. By boiling the infusion, I destroyed any germs there might have been in the liquid, or on the walls of the flask. But as this infusion remains in contact with the air, it undergoes alteration, like all such infusions.

But now suppose I repeat this experiment, but before boiling the liquid, I place the neck of the flask over a glazier's torch, allowing it to bend and stretch, while remaining open. I then boil the liquid, and allow it to cool. Now, the liquid in this second flask will remain completely unaltered, not just for two days, or three, or four, or even a month, a year, three years, or four! For the experiment just described has already been underway that long. The liquid remains completely pure, as clear as distilled water. What, then, is the difference between the two flasks? They both contain the same liquid, they both contain air, and they are both open. Why does one undergo alteration, while the other remains unchanged? Gentlemen, the only difference is this: In the case of the first flask, the germs contained in air-borne dust can fall down the neck of the flask, reaching the liquid, where they find appropriate nourishment, and proceed to develop. In the case of the second flask, however, it is impossible, or at least very difficult for air-borne dust to enter the flask, unless the air is extremely turbulent. Where does it go instead? It falls on the curved neck of the flask. When air enters the flask in accordance with the laws of diffusion, or as a result of relatively minor changes in temperature, it enters slowly, slowly enough that all of the dust and other solid particles it carries fall before they reach the opening, or along the early portions of the curved neck.

Gentlemen, this experiment is generous in its lessons. For notice that everything in the air, with the exception of dust, may enter the flask with extreme ease, thus coming into contact with the liquid. Whatever you imagine air to contain—electricity, magnetism, ozone, and perhaps even substances as yet unknown to us—it all passes through, reaching the infusion. The one thing which can't enter easily is dust, as demonstrated by the fact that, if I shake the flask violently two or three times, the infusion will, two or three days later, be seen to contain animalcules and mould.

Why? Because the rapid entry of air brought the infusion into contact with dust."

This excerpt is from a translation of notes for a speech given by Pasteur at the *Sorbonne Scientific Soirée* on April 7, 1864. This work was published in the journal *Revue des cours scientifiques de la France et de l'ètranger*, **1**(21), 23 avril 1864, 257–265. (Review of scientific studies in France and abroad, 23 April, 1864.)

Activity C3

Inquiry Lab

Student Reference 8

Required Skills
- Initiating and Planning
- Performing and Recording
- Analyzing and Interpreting
- Communication and Teamwork

Examining Pond Water

Until the late 19th century, there was a common belief that it was possible for life to spring from non-living matter. This belief in spontaneous generation arose in part because humans were unable to see the micro-organisms present in air and water. Pasteur's experiments provided strong evidence against spontaneous generation.

The Question

How many different organisms can be found in a sample of pond or aquarium water?

The Hypothesis

Write a statement predicting the variety of microscopic organisms present in samples taken at the surface, from the middle, and from the bottom of the container of pond or aquarium water provided by your teacher.

Materials and Equipment

pond or aquarium water
compound light microscope
3 glass slides and coverslips
tweezers or toothpicks
dropper
unlined paper
pencil
mathematical compass
unlined index cards
grease pencil or marker
glycerine or protoslo®
paper towel
chart paper

CAUTION: If any student in the class is taking immuno-suppressive drugs, micro-organisms should not be cultured in the classroom. Wash your hands at the beginning and end of this lab activity. Disinfect droppers, slides, and tweezers with a 10% bleach solution at the end of the activity. Dispose of equipment and clean your work area as directed by your teacher.

Procedure

1. Review Student Reference 8: The Compound Light Microscope, for the technique of making a wet mount slide.
2. Make 3 wet mount slides from the pond or aquarium water provided by your teacher. Take one sample from near the surface of the water, one from the middle, and one from the bottom of the container. Try to incorporate a small amount of debris in each wet mount. Label each slide with the part of the container from which it came.
3. Set up your microscope, and using the low-power objective, carefully examine each slide for signs of living organisms. Examine the organisms using the low-power objective lens.
4. Use the medium- and high-power objectives to study the organisms in more detail. Use the fine adjustment knob to bring the organisms into focus. Organisms may be swimming in and out of your field of view.
5. Add a drop of glycerine or protoslo® to the slide at the edge of the coverslip and draw it under the coverslip to slow down the movement of the organisms.
6. Draw a diagram of each of the organisms that you see. Place one diagram on each index card. Label any structures you recognize and record which part of the container the organism came from.

Analyzing and Interpreting

1. Did you detect differences between the 3 slides of samples taken from different parts of the container of water? What might this tell you about the presence of life at different levels in the container?
2. How many different types of organisms did you find? Do you think the variety of organisms may change over time if the water were to stay untouched for several days? Explain your answer.

Forming Conclusions

3. Write a statement to explain the diversity and quantity of microbes found in the pond or aquarium water.

Extending

4. How might an examination of pond water contribute to a consideration of the idea of spontaneous generation? Provide a reasonable argument that life cannot arise from non-living matter.

5. Using the diagrams on your index cards, sort the organisms by common characteristics. Determine your own criteria for classification. Compare your criteria and groupings with those of other lab groups. What similarities and differences were there in the classification systems? What similarities and differences were there in the organisms observed?

The Cell Theory

It was not until the 1830s, with the improvements in lens technology and the increased number of observations made by scientists in several countries, that the importance of the cell as the functional unit of life was recognized. In 1833, Scottish microscopist Robert Brown identified an important cell structure, the **nucleus**, in his study of orchids. Brown saw an opaque granular spot within the cell. Others before Brown had seen these spots, but he was the first to recognize that this cell structure must have some importance for cell function. Examine Figure C1.10 to see a picture of what Brown saw.

In 1838, a German professor of botany, M. J. Schleiden (Figure C1.11 (a)), observed that all plants were composed of cells and he proposed that the nucleus was in fact the structure responsible for the development of the remainder of the cell. Schleiden discussed his work with a friend, Theodor Schwann (Figure C1.11(b)), who was studying animal physiology. Schwann believed that there must be similarities between plant and animal tissue. When Schwann searched for the opaque spots in animal tissue, he found structures that resembled the cells that botanists were studying in plant tissue, and the nucleus structure that Brown and Schleiden had identified. He used his new-found information to put forward what has since been called the cell theory. Schwann and Schleiden proposed that all plants and animals were composed of cells and that the cell was the basic unit of all organisms. In 1859, the cell theory was further extended by Rudolf Virchow's statement that all cells arise only from pre-existing cells.

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Many of the organisms you observed in pond water are called "Protozoans." They are neither animals nor plants but belong to a separate kingdom, the Kingdom Protista. Some protozoans move by using a whip-like structure called a flagellum. Others move by using cilia, small hair-like projections along the sides of the cell that beat like tiny oars projecting them through the water. The amoeba, a jelly-like protozoan, moves by projecting a "false foot" or pseudopodium. Other protozoans are spore-like and have no method of locomotion.

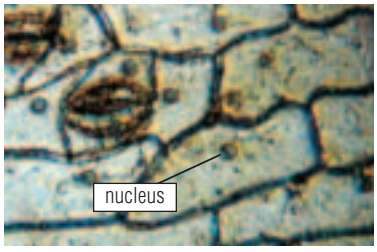

FIGURE C1.10 The nucleus is clearly shown in each cell in this photograph taken through Robert Brown's microscope. This preparation was made in a repeat of Brown's experiment and shows the view that he would have seen through his rather primitive instrument. (approx. ×200)

(a) Matthias Schleiden

(b) Theodor Schwann

FIGURE C1.11 Schleiden and Schwann proposed the cell theory in 1839 as a result of observations of plant and animal specimens through the microscope.

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Research other scientific contributions made by Louis Pasteur beyond his experiment to refute spontaneous generation. Begin your search at

www.pearsoned.ca/school/science10

The cell theory states:

- All living things are made up of one or more cells and the materials produced by these cells.
- All life functions take place in cells, making them the smallest unit of life.
- All cells are produced from pre-existing cells through the process of cell division.

We now know that the cell theory applies to all living things regardless of their size, shape, or the number of cells involved. Subcellular particles like viruses and prions fall into a category that is neither living nor non-living although they may exhibit certain characteristics of living cells. Evidence in support of the cell theory also came from Louis Pasteur's 1864 experiment to investigate the concept of spontaneous generation in micro-organisms, which was discussed earlier in this section. The cell theory has since become the cornerstone of the study of biology.

C1.2 Check and Reflect

Knowledge

1. Explain how the use of microscopes led to the development of the cell theory.
2. What is meant by spontaneous generation? Provide examples.
3. State the three components of the cell theory.
4. Describe the work of Louis Pasteur in refuting the theory of spontaneous generation.
5. The development of the cell theory from Hooke to Schleiden and Schwann was based on the very careful work of the scientists involved. What are the key components of scientific inquiry that are required for work to receive acknowledgement by the scientific community and society in general?

Applications

6. Create a visual representation to compare spontaneous generation and the cell theory.
7. Make a timeline of the experiments concerning spontaneous generation from Redi to Pasteur. Choose an appropriate format to present or display your work.
8. If straw is added to water, in a few days micro-organisms can be observed. Write one short paragraph in support of and another against the concept of spontaneous generation under these conditions.
9. Assume you are a scientist with the Canadian Space Agency analyzing data received from a distant space probe. There is a question about whether a certain material found on a new planet is living or not. List the characteristics that you will look for in your analysis to determine whether to classify the new substance as living.

Extension

10. Consider your findings from the examination of pond water. Design an experiment to test whether water temperature has an effect on the movement of the organisms.

C1.3 Developments in Imaging Technology and Staining Techniques

Light microscopes magnify cells through the use of one or more curved lenses and a light source. The compound light microscope is an important magnifying tool; research light microscopes have a maximum magnification of 1000–2000×. However, magnification is not the only factor that affects what can be seen through the microscope. Two other important aspects are contrast and resolution. You may have noticed when you examined organisms in your pond water preparation that it can be difficult to observe all of the cell structures because the organisms themselves are pale or transparent, and their background is equally pale. Any cell structures that you were able to distinguish were visible to you because more light was absorbed by that structure and it therefore appeared darker than its surroundings. The slight shading difference between components of the cell may be enough to distinguish them from one another, but not enough to observe subtle variations. As in art or photography, contrast is essential in order to see detail. Artists use lines, shading, and colour to create the illusion of three-dimensional images as seen in Figure C1.12.

The variation created by shading and colour allows the eye to focus on different aspects of the picture and to see depth. In the same way, scientists have perfected techniques to provide greater ability to see magnified objects in more detail. These technological advances allowed science to move ahead. Processes of innovation continue to meet the new challenges of inquiring minds and of new problems to be solved.

Contrast

When light passes directly through cells in what is called **brightfield** microscopy, most of the cells seen are colourless. Scientists quickly discovered that manipulating the light source could alter the contrast between structures in the cell and improve the image. Experiments with stains or

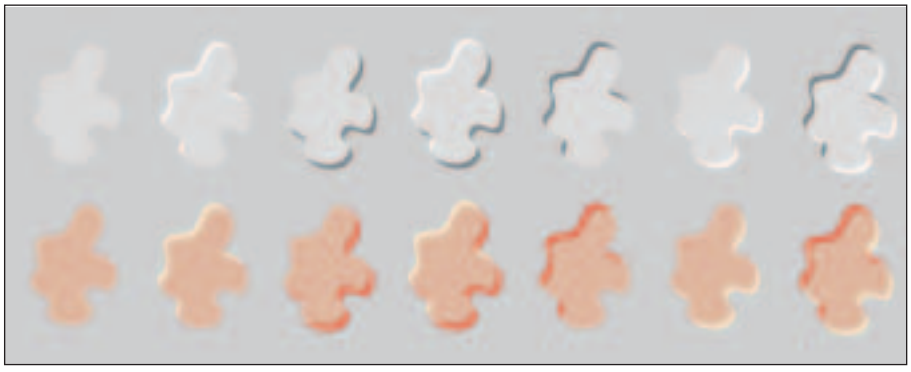

FIGURE C1.12 These images demonstrate the effect of different levels of contrast.

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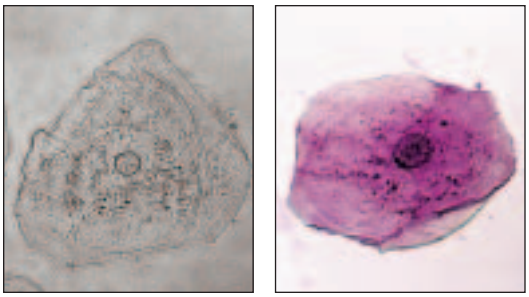
Digital photographic images can be enhanced simply by manipulating the colour or shading of the background to produce more contrast. If you have a digital photograph of people standing in front of a wall, try changing the shading or colour of the wall and see what happens to the image.



(a) Unstained (approx. ×1500) (b) Stained (approx. ×1500)

FIGURE C1.13 A comparison of human cheek cells as seen through the light microscope

colouring agents showed that particular stains attached to particular parts of the cell, improving the contrast between internal structures and producing better images. Staining techniques can be as simple as adding methylene blue or iodine stain to a specimen. Chemical preservatives "fix" the cells and allow more complex staining procedures. We now know that the staining properties depend on the chemical composition of the structures in the cell. One disadvantage of these techniques is that fixation and staining kill the cells, so it is not possible to view living tissue. Figures C1.13 and C1.14 (on page 255) show the detail to be seen when different stains are used.

Activity C4

QuickLab

Student Reference 8

Staining Cells

Purpose

To use a staining technique to provide contrast in order to observe plant cells

CAUTION: Handle glass slides and coverslips carefully. Remember that biological stains also stain clothing. Wear disposable gloves during the activity. Wash your hands at the end of the lab activity.

Materials and Equipment

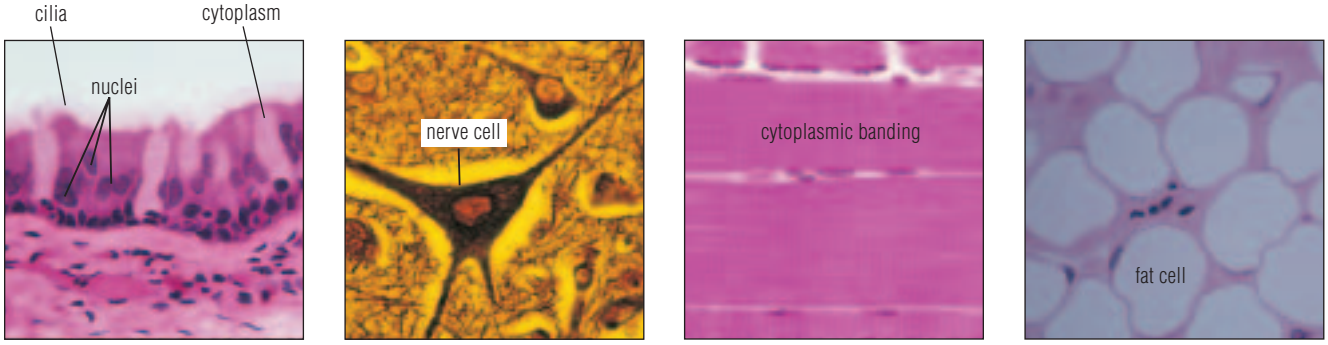
compound light microscope
4 glass slides
4 coverslips
flat wooden toothpicks
stains: iodine, and methylene blue
droppers
paper towel or tissues
yellow or white onion

Procedure

1. Review Student Reference 8 and follow the directions when making a wet mount preparation, drawing a scientific diagram, and calculating the scale of a scientific diagram.
2. Remove the dry, outermost layers from an onion and use a thin, transparent layer of the onion to get one layer of cells. You may stain the onion with iodine or with methylene blue. Prepare a wet mount slide.
3. Under low magnification, observe the cells you have stained. Draw and label what you see in the field of view.
4. Examine your slide further using the medium- and high-power objectives. What are you now able to see that you could not see with the low-power magnification?
5. Estimate the size of the onion cells by comparing them with the diameter of the field of view that you determined in Activity C2. Draw and label the cells. Indicate the magnification used and the scale on each drawing. Compare your preparation of onion cells with that of a student who has used a different stain.

Questions

1. Describe the cells present in your preparations.
2. Compare what you saw in the unstained pond water organisms to what you saw in these stained cells. What advantages are gained by using stains?
3. What differences are observed at the different magnifications? Describe them.
4. What was the effect of using a different stain on the onion cells?



(a) The epithelial cells have cilia on the surface. Their nuclei (plural for nucleus) stain more deeply purple than their cytoplasm. (approx. ×120)

(b) The nerve cell stains dark brown. (approx. ×120)

(c) Cells in one type of muscle tissue show a clear pattern of banding in the cytoplasm. (approx. ×250)

(d) Cells that contain fat droplets in living tissue appear colourless in stained preparations because the fixation process dissolves the lipids. (approx. ×120)

FIGURE C1.14 Staining techniques have been developed to highlight certain parts of cells seen through the light microscope.

Resolution

Magnification is an important component of the microscope but it is not enough simply to magnify an object. There are other aspects that are important to image formation. Hold two pencils or pens close together upright directly in front of you. Make a mental note of the detail that you observe. Now move the pencils to one side. If you kept looking straight ahead, you could probably still see the pencils from the corner of your eyes. However, you probably could not see the images of two pencils distinctly. **Resolution**, or **resolving power**, is the ability to distinguish between two structures that are very close together. You get better resolution when you look at something directly in front of you than when you look from the corner of your eyes. Resolution is an important aspect of any image—an image should have high resolution if it is to show its details.

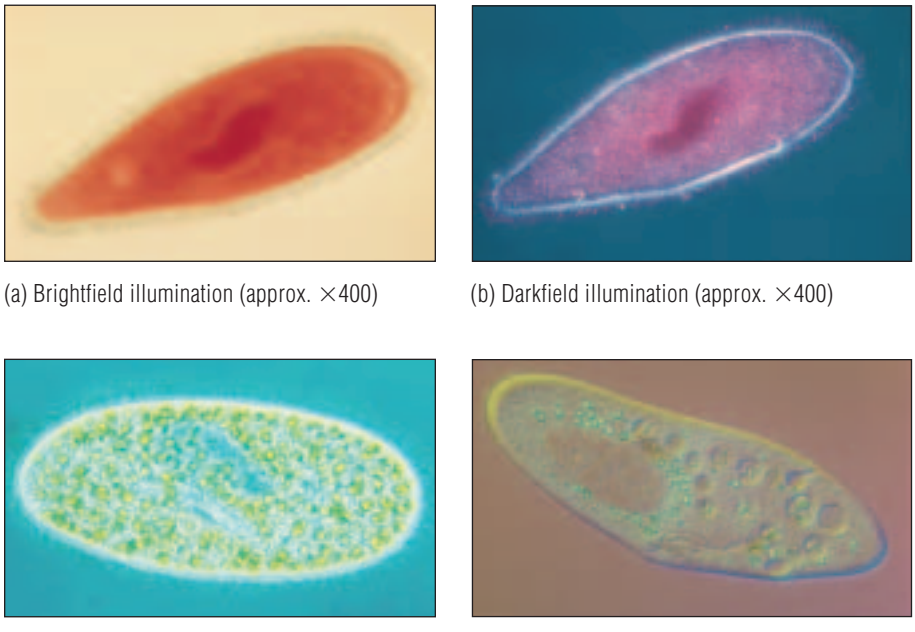
Look at the images in Figure C1.15. The images are of the same object but the clarity is very different. The first one is fuzzy and difficult to decipher. You may even be able to see individual dots or squares of colour, called pixels. Pixels are used in creating the images you see on your computer monitor or from a digital camera. The second one provides a more clearly defined view. In this image, the pixels are smaller and there are more of them per unit area than in the first one. The second image has a higher resolution and you can see the details more clearly.

FIGURE C1.15 The resolution of the image can make a great difference in the amount of detail seen.

The human eye can distinguish images of objects that are 0.1 mm (1×10^{-4} m) or larger. A microscope must provide the capability to see anything smaller than this, or to see individual objects that might be closer together than 0.1 mm. Regardless of the level of magnification, the clarity of images depends on the resolving power of the microscope. The efficiency of the light microscope is limited because as light is focussed into smaller and smaller diameters, the image becomes blurred. Because light microscopes depend on white light illumination, and even with the improvements in lens making made during the 19th century, the resolving power of the light microscope is limited by the wavelength of light. Points closer together than one-half the wavelength of light will not be seen as separate. The limit of resolution of a standard light microscope is 0.2 μm (2×10^{-7} m).

Contrast Enhancing Techniques and Fluorescence Microscopy

As science progresses, it tends to reach the limits of what can be accomplished with available technology and so pushes the limits of that technology. If innovations in technology are not available, progress may be limited. Some structures in cells can change the speed and direction of light passing through them more than others can. In the first half of the 20th century, techniques were developed to improve images by altering the light path through the specimen. These included darkfield, phase contrast, and differential interference contrast illumination. Figures C1.16(a)–(d) show images of *Paramecium* under different systems of illumination. Fluorescence microscopy was developed and gave information about molecules on the cell surface. With this technique, fluorescent substances that



(a) Brightfield illumination (approx. ×400)

(b) Darkfield illumination (approx. ×400)

(c) Phase contrast illumination (approx. ×250)

(d) Differential interference contrast illumination (approx. ×250)

FIGURE C1.16 Images of *Paramecium* seen through the light microscope under different systems of illumination

were attached to molecules in tissues could be located. When the specimen was subjected to ultraviolet light, the fluorescent molecule emitted light of a different wavelength, causing it to glow. Depending on the fluorescent substance being used, the glow could be yellow, orange, or green. In 1941, J. H. Coons used antibody molecules labelled with a fluorescent substance to show that antigens were on the surface of cells. The molecules that determine blood groups in humans are examples of cell surface antigens.

Confocal Technology

Since the 1980s, the use of laser beams and computers has made it possible to view living, transparent cells in three dimensions through the compound light microscope. In the **confocal microscope**, a laser concentrates light onto a specimen. The reflection is passed through a tiny opening called the confocal pinhole and reaches an electronic detector that converts the light into an image. Only the light returning from an exact plane of focus can pass through the pinhole to the detector because, as Figure C1.17 shows, out of focus light is blocked by the edges of the pinhole. Therefore, every image formed is of a very thin section through the specimen. Each image is stored in a computer and images of many sections are combined to produce a three-dimensional image that can be viewed on a computer monitor. Figure C1.18 shows a worm that is used in research under confocal laser scanning microscopy.

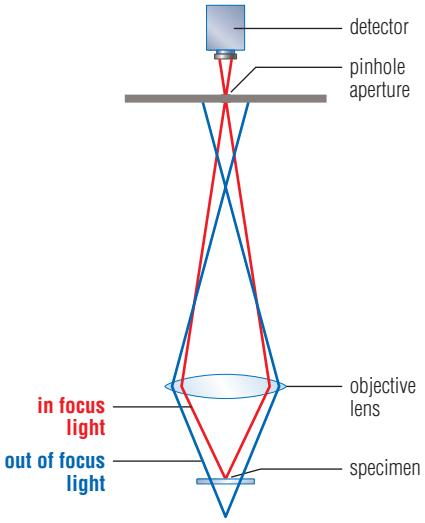


FIGURE C1.17 In a confocal system for the light microscope, only light from any part of the specimen that is in focus will pass through the pinhole to the electronic detector. Out of focus light will be blocked by the sides of the pinhole and not reach the detector. This produces the effect of a thin section with high resolution. Many sections can be combined to produce a three-dimensional image.

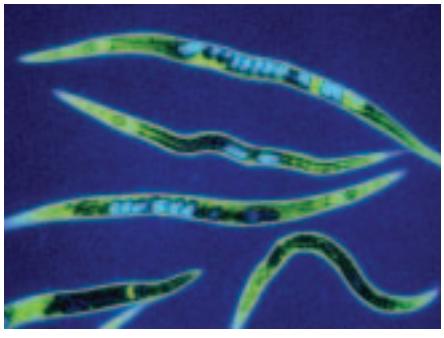
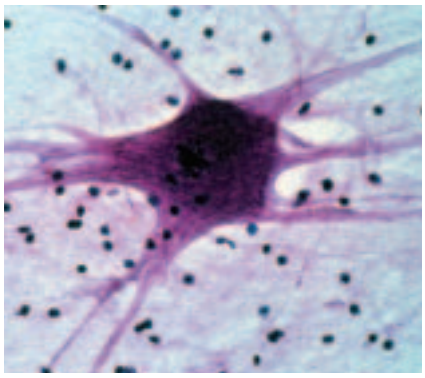


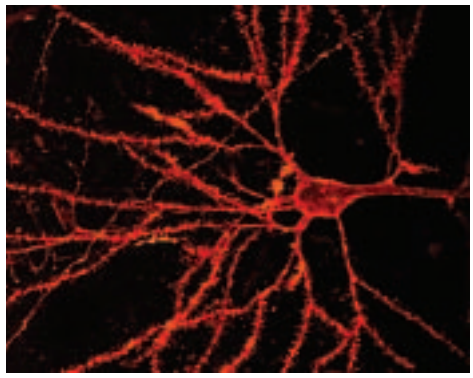
FIGURE C1.18
A nematode worm viewed under confocal laser scanning microscopy. The round internal structures are eggs. (approx. ×80)

reSEARCH

Find out more about the various types of contrast enhancing techniques used to improve the detail of microscopic images. Begin your search at www.pearsoned.ca/school/science10



(a) A motor neuron under brightfield illumination (approx. ×250)



(b) A brain neuron fluorescing under ultraviolet light, seen through confocal laser scanning microscopy

FIGURE C1.19 Nerve cells under different forms of illumination

Figures C1.19(a) and C1.19(b) compare images of nerve cells obtained using brightfield illumination and confocal technology. Under brightfield illumination the processes called dendrites that bring information to the cell body of the motor neuron merely appear fibrous, but, using confocal technology, the finely branched three-dimensional structure of the brain neuron dendrites can be seen.

The confocal microscope gives opportunities for the use of other techniques to study the cell. Among these is the use of the green fluorescent protein (GFP), first found in a luminescent jellyfish called *Aequorea victoria*, shown on the opening pages of this unit. This protein glows a bright green when exposed to ultraviolet light, in the same way that fluorescent paint glows in the presence of black light. Scientists have been able to attach GFP to certain parts of cells that they wish to study, almost like a tiny lamp! While staining methods kill the cell, the use of fluorescence microscopy with GFP allows the study of living cells in a way that was not possible prior to current technology.

Electron Microscopy

In the first half of the 20th century, as scientists struggled to improve light microscopes, researchers were also looking for ways to improve the resolution of imaging systems. They turned to forms of illumination other than light. In the 1930s at the University of Toronto, James Hillier and Albert Prebus developed the first functional **electron microscope**. An electron microscope uses a beam of electrons instead of a light wave and is able to produce images that provide fine detail. The image is formed by the absorption or scattering of the electron beam because **electron-dense** materials do not let the electrons pass through. Focussing is done by adjustment of electromagnets instead of by movement of glass lenses.

The **Transmission Electron Microscope (TEM)** depends on a beam of electrons passed through a very thin section of fixed and stained tissue embedded in plastic. The electrons that pass through the specimen fall on a fluorescent screen (see Figure C1.20) or on photographic film and black-and-white photographs are produced.

The TEM may operate at magnifications of up to 1 500 000× and has a resolution for biological specimens of about 2.5 nm. This is an improvement

of one-hundred-times over the detail shown in the light microscope. Some aspects of image formation in the light and electron microscopes are compared in Table C1.1.

TABLE C1.1 Comparison of Light and Transmission Electron Microscopes

Feature	Light Microscope	Electron Microscope
source	lamp or laser	electron gun
radiation	UV or visible light	electron beam
lenses	curved glass surfaces	electromagnets
receiver	eye or digital image	fluorescent screen or digital image
focus	up and down movement of lenses	adjustment of magnetic field

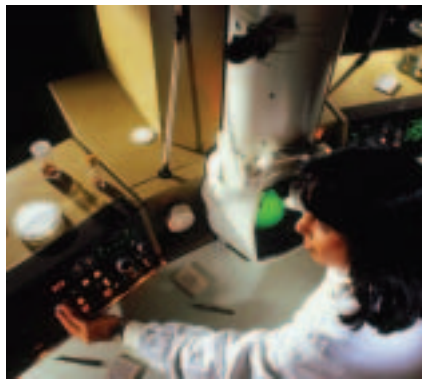


FIGURE C1.20 Electrons travelling down the microscope column pass through the specimen and an image forms on a fluorescent screen at the bottom of the column. The scientist views the image on the screen.

The **Scanning Electron Microscope (SEM)** was developed in the 1940s. This technology gives information about the surface features of a specimen. Specimens are fixed and covered with an electron-dense material like gold, which reflects electrons. While the investigator scans the surface of the specimen, the electrons bouncing off the surface are picked up by a sensor and a three-dimensional image is formed. Figure C1.21 shows an SEM view of *Paramecium*. The SEM operates up to a magnification of 300 000× and has a resolution of 20 nm. The image may be viewed on a screen or captured on computer and viewed on a monitor. The investigator can move the specimen in three dimensions with coarse and fine adjustments or the sample stage may be computer controlled (Figure C1.22).

Photographs taken through either scanning or transmission electron microscopes are called electron micrographs. They provide a detailed view of the cell's surface texture, the shape and size of the particles in the cell, and how the materials are arranged. Electron microscopes provided more detailed images of cell structures that were already known, such as the nuclear envelope and Golgi apparatus. Electron micrographs also showed

FIGURE C1.21 The surface of *Paramecium* is covered by cilia, short hair-like structures, used for swimming and wafting food into the groove-like mouth. (approx. ×300)

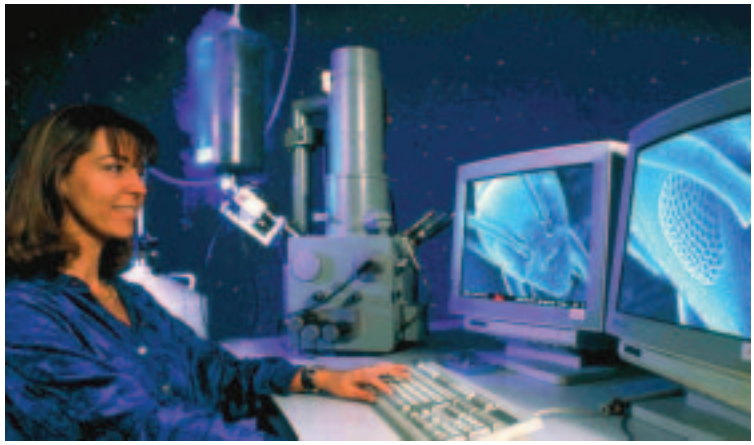


FIGURE C1.22 The electron beam coming down the microscope column sweeps over the gold-coated specimen and a three-dimensional image is formed.